1417-Pos Board B187
Measuring the Plasmid Copy Number of Single E. Coli by Digital PCR: Are Plasmids Partitioned in Clusters during Cell Division?
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The presence of plasmids is important for the behavior of bacteria, as they often carry antibiotic resistance genes and genes involved in virulence of pathogenic strains. Furthermore plasmids are often responsible for horizontal gene transfer. In order to remain in the bacteria the plasmids have to provide complex regulation mechanisms and often inhere active partitioning during cell division. Fingerprints of the resulting plasmid stability can be found in the plasmid copy number distribution. However, these distributions have been rarely reported, since they have been experimentally difficult to obtain.
We performed single cell measurements that allow to piece together the plasmid copy number distribution in a bacterial population. We use fluorescent activated cell sorting (FACS) to isolate single bacteria and subsequently utilize the single molecule resolution of digital PCR to determine the plasmid copy number of single cells.
Interestingly, the measurement of the plasmid pZE which has a ColE1 like origin of replication showed a rather broad distribution. An unexpectedly high percentage of the cells had very low plasmid copy numbers, despite the high mean copy number of 120. This characteristic however disappeared for a mutant which additionally contains the active partitioning system parABC. The instability of the pZE copy number is unlikely to be the result of random plasmid partitioning; we explain the findings by clustered partitioning, i.e. clusters of plasmids are partitioned to the daughter cells rather than the individual plasmids. Results are high statistical fluctuations of the plasmid copy number. Other groups showed evidence for clustering of plasmids in E. Coli and also showed, that these clusters get dissolved if the plasmids contain parABC genes. Furthermore, theoretical models which employ clustered partitioning fits our data quantitatively.

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DNA Nanoprobe for Intracellular Dynamics
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We have developed a novel method to follow the dynamics of DNA interacting with the cellular environment in vivo using two-color correlation microscopy. A DNA probe is end-labeled with two quantum dots and transfected into an axenic strain of Dictyostelium discoideum. The motion of the quantum dots is observed with two-color fluorescence video microscopy. The computed time correlation functions of this two-particle motion reflect the fluctuations of the DNA probe as a result of its interactions with the cellular environment. Substantial differences between live cells and dead yet structurally intact cells point to a strong coupling of active, motor-driven fluctuations in the cell to the DNA probe. This suggests that the motion of native cellular DNA may similarly be driven by active processes instead of relying on purely thermal passive fluctuations. We also note that the difference between the autocorrelations of the center of mass motion and the relative motion of the two quantum dots is a sensitive measure for the effective length of the DNA probe on a length scale around one persistence length (~ 50 nm). This paves the way for experiments with more complex DNA probes that can bind to intracellular proteins, and report single-molecule binding events through apparent length changes and consequently changes in this correlation measure.

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Single Molecule Live Cell Millisecond Fluorescence Imaging of Bacterial Condensins
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The MukBEF protein complex in Escherichia coli is a condensin-like system that binds to bacterial chromosomal DNA. Using bespoke slimfield millisecond fluorescence imaging we were able to visualize the three MukB, E and F protein components in functional complexes in single live cells, both separately as genomic YPet fusions and multiply as dual-label GFP/mCherry mutants and obtain in vivo estimates for the stoichiometry of these components using stepwise photobleaching of fluorescent proteins, precise down to the level of single molecules. This indicates a predominately tetrameric complex of B:E:F = 4:4:2 suggesting that the DNA-bound MukBEF in the live cell is in its ATP-bound state, and which is highly dynamic in the living cell with focal complexes undergoing molecular turnover over a time scale of tens of seconds. The dynamic localization observed is consistent with a spontaneous oscillation-type model which may be used in the essential positioning of replicated DNA prior to successful DNA segmentation into newly divided cells.

1420-Pos Board B190
Structure and Dynamics of Supercoiled DNA in Confinement
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Cells must package their chromosomes within a volume whose characteristic length is orders of magnitude smaller than the genomic length. When sufficiently twisted, DNA assumes a supercoiled form, aiding in this necessary compaction, as suggested in the case of Caulobacter crescentus. Given the crucial role supercoiling plays in bacterial DNA packaging, an elucidation of supercoiling structure and dynamics under confinement promises to shed light on critical living processes during replication as well as genome accessibility during transcription. We explore the structure and dynamics of supercoiled DNA by modeling the strand as a self-interacting, elastic beaded chain with twist and conduct Brownian Dynamics simulations under various geometries and degrees of confinement. The mean end-to-end distance between DNA segments exhibits a scaling of 0.25 with genome contour length, consistent with recent experiments in Caulobacter and suggesting a randomly branched supercoiled configuration. The scaling subsequently becomes linear at lengths comparable to the size of the enclosure. The mean-distance dynamic behavior at various length scales is highly sensitive to degree of confinement, and we identify the biologically relevant time scales of relaxation. Short genome lengths relax relatively fast with power-law scaling at all confinements while highly confined DNA is impeded from fully exploring its configurational space, resulting in delayed relaxation at large length scales. In this regime, the DNA segments become effectively quenched, resembling a stiff, glassy state that prohibits large-scale movement. We delineate the length-dependent transitions between these dynamic states and discuss their biological consequences in light of genome accessibility and reorganization.

1421-Pos Board B191
Force Fluctuations Impact Genome Processing Kinetics
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The current view of force-dependent enzyme kinetics neglects the role of fluctuations in the force, which are prevalent in all living cells due to thermal noise and active processes. Our calculations indicate that these fluctuations can qualitatively alter the dynamics of vital biomolecular processes, including transcription and supercoiling maintenance. As an illustrative example, we model the pausing of RNA polymerase as it transcribes through a nucleosome, demonstrating the importance of transient excursions to high forces exerted by fluctuations in wrapping of downstream DNA. We find that fluctuation timescales play a key role in setting overall transition rates and determinining the force regime of relevance. In addition, we consider the maintenance of DNA supercoiling by topoisomerase enzymes acting in a torsion-dependent manner. Using a basic kinetic model incorporating discrete torsion fluctuations and volatile obstacles, we show a nontrivial dependence of the equilibrium supercoil density on the length of DNA accessible for twist propagation. Our results demonstrate that accounting for force fluctuations is essential to developing a quantitative understanding of enzyme kinetics in the dynamic environment of a living cell.

1422-Pos Board B192
RNA Biophysics in Living Cells using Shape Chemistry
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Current knowledge of RNA structure and function has primarily been derived from studies carried out in dilute solution. However, the normal environment for RNA in cells is a highly crowded one, in which the concentration of macromolecules reaches 300 g/L. Global effects of synthetic crowding agents, such as polyethylene glycol, indicate that crowding can have dramatic effects on RNA structure. Studies under physiological conditions and at the level of individual nucleotides, however, are required to fully understand the biological structure-function relationships involving RNA. In this study, we use selective 2'-hydroxyl acylation analyzed by primer extension (SHAPE) to probe the structure of a purine riboswitch RNA aptamer domain in healthy growing *Escherichia coli* cells. Our results identify nucleotide-resolution effects of the cytoplasm on RNA structure and show that the cellular environment indeed alters the structural properties of the purine riboswitch relative to dilute solution. Specifically, significant decreases in SHAPE reactivity are seen in the purine binding pocket in cells compared to dilute solution. We conclude that in cells, the purine aptamer exists in a less flexible, more structured conformation. Such structural differences can have consequences on ligand binding properties, such as equilibrium dissociation constants. Ultimately, our results show that dilute solution experiments only partially recapitulate the cellular environment and it is critical to consider the effects of macromolecular crowding to accurately understand RNA structure and function in cells.